

Investigation of the traditional enzymatic role and the emerging regulatory function of dUTPases

Theses of the Ph.D. dissertation

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1 INTRODUCTION

My thesis focuses on the enzymatic properties of the trimeric dUTP pyrophosphatase and the interconnections of these properties with the traditional and regulatory functions of dUTPase. The dUTPase enzyme belongs to the dUTPase superfamily, which comprises also the dCTP deaminase (DCD) and the bifunctional dCTP deaminase-dUTPase (DCD-DUT) enzymes. The members of this superfamily produce important precursors for dTTP biosynthesis. In addition, dUTPase also has a preventive DNA repair role by the removal of dUTP from the dNTP pool which prevents dUTP incorporation into DNA.

dUTPase, DCDs and DCD-DUTs share a common homotrimeric structural core¹⁻⁴. The three subunits form a central channel and three equivalent active sites at the intersubunit clefts. This intricate quaternary structure intuitively suggests the possibility for allosteric control within the enzyme. In effect, the allosteric communication between the active sites of DCD and DCD-DUT family enzymes has been investigated in several species^{3,5-7} and was found to operate through the central channel^{3,7}. dUTPases display an even more intricate interaction pattern between their subunits than DCDs and DCD-DUTs do. Their C-terminal part reaches across the trimer to the remote active site and therefore, all three subunits contribute conserved residues to each active site⁴. This structure inspired the proposition that allosteric communication between the active sites of dUTPases should also exist⁸⁻¹⁰.

Beside their metabolic and preventive DNA repair role, dUTPases participate in several additional processes, including the activation of the horizontal gene transfer (HGT) of *Staphylococcus aureus* pathogenicity islands (SaPI). SaPIs themselves do not encode any machinery for HGT, they take advantage of phage reproduction instead¹¹. In the absence of a helper phage, the expression of SaPI-encoded transfer initiating proteins (integrase and excisionase¹²) is repressed by SaPI-encoded repressor proteins called Stl. Helper phage infection or prophage activation relieves Stl repression and leads to the excision and extensive replication of SaPI DNA that is subsequently packaged into phage capsids¹¹. In case of SaPI_{bov5} and SaPI_{bov1} it was found that they are de-repressed by dUTPases from phage 80 α (for both) and phage Φ 11 (for SaPI_{bov1})^{13,14}. Here, the enzymatic cycle of dUTPase was suggested to play a key role in the activation process. Moreover, in this process the dUTPase-regulated gene transfer was proposed to adopt a mechanism highly similar to G protein mediated signaling, where the switching conformational change between the on/off states occurs upon GTP binding to the G protein¹³.

dUTPases play key roles both in the maintenance of the appropriate dUTP/dTTP ratio and in various additional biological processes. No surprise that dUTPases are investigated as potential drug targets. Understanding the exact role and mechanism of this enzyme is essential.

In this study, we had two main goals:

- I. We set out to investigate the manifestation of allostery within the superfamily of dUTPases. We attempted to detect allosteric behavior in dUTPases by engineering hybrid enzymes (Figure 1) to restrict putative allosteric transmission between active sites at various stages of the enzymatic cycle. Besides, we also aimed to investigate the characteristics of the central channel of dUTPases to explain the manifestation of allosteric behavior within the dUTPase superfamily.

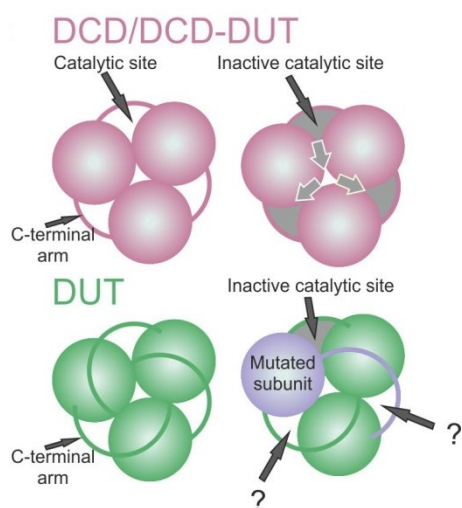


Figure 1

Schematic representation of the potential active site communication within dUTPase, based on the example of DCD-s and DCD-DUTs

Grey color at the active sites represents inactive catalytic site. In case of DCD (-DUT) arrows in the central channel show that the conformational switch is transformed through the central channel in a concerted way. Note that in case of dUTPase the C-terminal arms are reaching out from one subunit to contact the remote active site.

- II. dUTPase and its enzymatic function were shown to play a key role in SaPI HGT activation^{13,14}. A G protein-like mechanism was proposed including a dUTP induced conformational change as key switch between the active and inactive states of dUTPase. However, the interconnection of the enzymatic cycle and the de-repressing function was not studied.
 - a. We aimed to characterize the enzymatic properties of Φ 11 dUTPase and its interaction with the StI protein.
 - b. We also wished to explore how the dUTP substrate and the StI protein influence the binding of each other to dUTPase.

3 METHODS

For Aim I, we designed mutations to access inactive conformational states in dUTPase potentially mimicking the ones observed in DCD and DCD-DUT. To this end, we created covalently linked human dUTPase pseudoheterotrimers (called hybrids henceforth) in which the active sites could be turned off selectively. We studied how the interruption of different steps of the enzyme cycle in one active site of the hybrid affects the non-mutated active sites using various enzymatic and structural biology approaches, such as steady-state and transient kinetics, circular dichroism spectroscopy, ThermoFluor assay and limited trypsinolysis. Our most powerful tool for the kinetic characterization was a tryptophan sensor engineered to the active site of the investigated dUTPases^{15,16}. With this active site sensor we could investigate the detailed kinetic properties of the different dUTPase variants using fluorescence stopped-flow¹⁷.

For Aim II, we used similar enzymatic and structural biology approaches. Luckily, the same active site tryptophan could also sense the binding of the Stl protein to phage dUTPase, which allowed us to perform the kinetic characterization of the complex formation of dUTPase with dUTP and Stl. Besides, quartz crystal microbalance method and used electrophoretic shift assay were also used. To check whether *S. aureus* strains contain dUTPases, we used bioinformatic tools.

4 RESULTS AND CONCLUSIONS

RESULT AND CONCLUSIONS FOR AIM 1

We created hybrid human dUTPases to study possible other means of cooperativity in detail. In these hybrid enzymes one or two monomers **a**, were catalytically inactive **b**, had low activity and reduced substrate affinity due the lack of the C-terminal swapping arm **c**, or did not bind substrate at all. Using these asymmetric hybrids, we investigated whether the interruption of different steps of the enzyme cycle – and thereby the interruption of the possible conformational changes occurring during these steps – affects the functioning of the non-mutated active sites. We also investigated the central channel.

We determined the followings:

- (i) The active sites work independently from each other.
- (ii) Mg^{2+} binding in the central channel reduces the flexibility and increases the thermal stability of the quaternary structure.
- (iii) The allosteric loop that connects the active site to the central channel is conformationally restricted in dUTPases compared to DCD family enzymes. This phenomenon is interrelated with structural solutions for increased dUTP specificity in every case.

We propose that the trade-off between cooperativity and specificity in the dUTPase superfamily represents instances of adaptation to the distinct roles of dUMP production for dTTP synthesis and dUTP elimination for uracil-DNA avoidance, respectively.

RESULT AND CONCLUSIONS FOR AIM 2

Investigating the $\Phi 11$ phage dUTPase driven SaPI HGT we have found the following:

- (i) $\Phi 11$ phage dUTPase is a highly efficient dUTPase which hydrolyzes dUTP with a kinetic mechanism fundamentally similar to the kinetic mechanism described earlier for other dUTPases.
- (ii) Stl is a slow and tight binding, competitive inhibitor of the $\Phi 11$ dUTPase, which contradicts the earlier proposed G protein-like mechanism of this molecular switch.
- (iii) The sequenced *S. aureus* strains contain dUTPases only as part of integrated prophages, which are not expected to be expressed in lysogenic phase, therefore the dUTP level is probably high within the bacterium.

Our results allowed setting up a molecular model for the activation of SaPI HGT by dUTPases. In this model, the phage dUTPase is able to interact with Stl only after sanitizing the dNTP pool from dUTP thereby promoting the uracil free replication of the excised SaPI DNA.

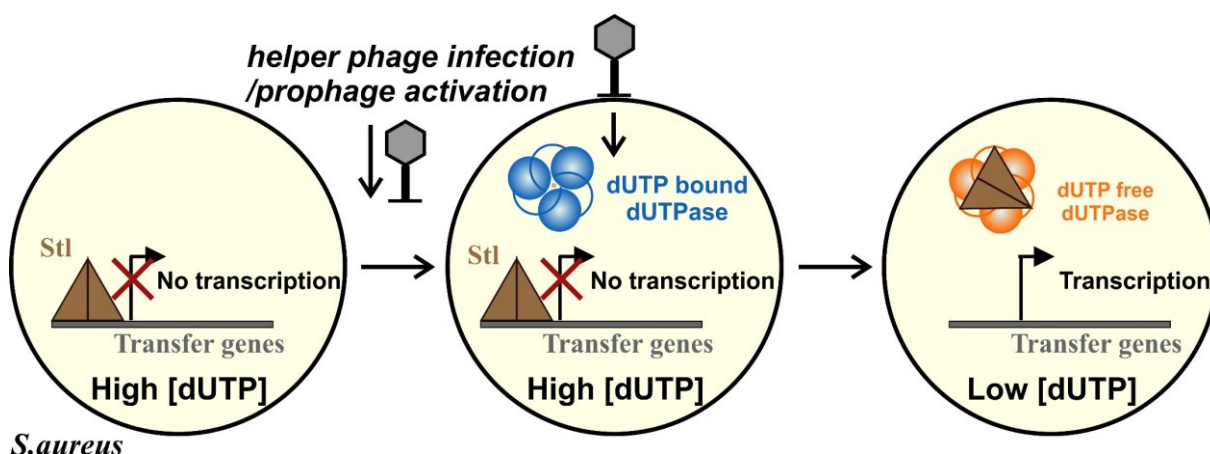


Figure 2

Model of the mechanism of dUTPase-controlled SaPI activation

Besides the understanding of such an important mechanism like the HGT of pathogenicity islands, it is also important that we identified Stl as the first, highly efficient protein inhibitor of dUTPase. If Stl proves to be a general dUTPase inhibitor – which it seems to be¹⁸ – it can ease the clarification of the various roles of dUTPases in different species *in vivo*.

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6 PUBLICATIONS RELATED TO THE DOCTORAL THESIS

PUBLICATIONS CLOSELY RELATED TO THE THESIS

PEER-REVIEWED PUBLICATIONS RELATED TO THE THESIS

- 2014 **Szabó, J. E.**, Németh, V., Papp-Kádár, V., Nyíri, K., Leveles, I., Bendes, A. Á., Zagyva, I., Róna, G., Pálinkás, H. L., Besztercei, B., Ozohanics, O., Vékey, K., Liliom, K., Tóth, J., and Vértessy, B. G. (2014) Highly potent dUTPase inhibition by a bacterial repressor protein reveals a novel mechanism for gene expression control. *Nucleic Acids Res.* 42, 11912–20.
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- 2015 **Szabó, J.E.**, Takács, E., Merényi, G., Vértessy, B.; Tóth, J. Trading in cooperativity for specificity to maintain uracil-free DNA. *Submitted to eLife*.

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ORAL PRESENTATIONS – PRESENTING AUTHOR UNDERLINED

- 2015 **Szabó, J.E.**, Hirmondó, R., Dobrotka, P., Tarjányi, Sz., Nyíri, K., Tóth, J., Vértessy, B.G., *Characterization of species-specific inhibitory effect of a Staphylococcal repressor protein on dUTPases*. FEBS3+ meeting; Portorož, Slovenia
- 2015 **Szabó, J.E.**, Nyíri, K., Hirmondó, R., Papp-Kádár, V., Dobay, O., Szabó, D., Tóth, J., Vértessy, B. G. *A Novel Mechanism Provides Genome Quality Control For Pathogenicity Island Transfer In Staphylococcus*. 17th International Congress of the Hungarian Society for Microbiology, Budapest, Hungary
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POSTER PRESENTATIONS – PRESENTING AUTHOR UNDERLINED

- 2015 **J. E. Szabó**, P. Dobrotka, J. Tóth, B. G. Vértessy *Characterization of species-specific inhibitory effect on dUTPases by a transcription factor encoded by Staphylococcus aureus*. Hungarian Molecular Life Sciences; Eger, Hungary.
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